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Chemistry and Physics of Lipids 133 (2005) 215–223
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Short communication

Synthesis and preliminary physical applications of a rhodamin-biotin phosphatidylethanolamine, an easy attainable lipid double probe

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Received 14 April 2004; received in revised form 25 October 2004; accepted 25 October 2004
Available online 26 November 2004

Abstract

In route to a physical study aimed at understanding lipids and proteins sorting in cells, we designed a rhodamin-labelled biotinylated phosphatidylethanolamine (PE), as a useful and easy-attainable lipid double probe. The target compound was successfully engaged in preliminary physical experiments.

Keywords: Synthesis; Rhodamin; Biotin; Phosphatidylethanolamine; Lipid probe

1. Introduction

Many physical or biological studies of cell events require the preparation of unilamellar vesicles (UV) mimicking biological membranes. Thus, synthetic UVs (as liposomes) are totally controlled minimal systems aimed at characterizing how membranes work, both qualitatively and quantitatively. These studies can be accomplished by anchorage of peptidic cytoplasmic recognition patterns (Leborgne et al., 1998), adhesion motifs (Hojo et al., 1996) receptor ligands (Sugimoto et al., 1995; Fukasawa et al., 1998) or enzymes (Bartz et al., 2003) into the UV lipid bilayers. Regarding this

Abbreviations: Boc, tertio-butyloxycarbonyl; DBU, 1,8-diazabicyclo[5,4,0]undec-7-ene; DHB, 2,5-dihydroxybenzoic acid; DIEA, diisopropyl ethyl amine; DMAP, 4-*N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DSPE, distearyl-phosphatidylethanolamine; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *N*-[1H-(benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methyl methanaminium hexafluorophosphate *N*-oxide; HOBt, *N*-hydroxybenzotriazole; NMP, *N*-methylpyrrolidone; Pam, palmitoyl; PyBop, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; TMCR, 5,6-tetramethylcarboxyrhodamin; TFA, trifluoroacetic acid; TIS, triisopropylsilane; TNBS, 2,4,6-trinitrobenzenesulfonic acid

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0009-3084/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2004.10.005

type of immobilisation, the partition of biomolecules modified by long alkyl chains between membranes and solutions have been extensively studied (Epand, 1997). They showed that a stable insertion requires at least two proximal alkyl chains. However, the lipidation of hydrophilic compounds is often troublesome due to the generally low solubility of the conjugates. To circumvent these difficulties, convergent strategies have been developed where the purified biomolecule is bound to a functionalized lipid anchor already inserted into the membrane. Covalent bonds are chosen among amide function (Kung and Redeman, 1986), thioether bond (Boeckler et al., 1998; Heeremans et al., 1992; Kamps et al., 1996; Martin and Papahadjopoulos, 1982; Schelte et al., 2000), or disulfide bridge (Barbet et al., 1981), whereas non-covalent links are usually obtained by the avidin/biotin couple (Loughrey et al., 1993) or Ni^{2+}/histidine complex (Celia et al., 1999).

For studying lipids and proteins sorting in cells, we designed a minimal system mimicking the formation of tubular structures from intracellular compartments (Roux et al., 2002). In this system, 'molecular motors', as kinesin, had to be anchored to a giant UV (GUV) outer membrane, via a biotin/avidin link. Moreover, the presence of a fluorescent probe was necessary to trace any event occurring in the membrane (deformation, budding, tubular formation, raft occurrence...) and unambiguously correlate it to the presence of kinesin. So, we searched for an available phospholipid anchor that would bear both a biotin for the motor attachment and a fluorescent probe.

Commercially available functionalised phospholipids (or described in the literature) usually bear either a biotin or a fluorophore on the hydrophilic head. Some of them contain both a biotin and a fluorophore, but in this particular case, the fluorophore replaces a fatty acyl chain (Roodsari et al., 1999; Davenpport et al., 2001), they cannot be used when the two fatty chains are required (Blumenthal et al., 2002; Maier et al., 2002).

In addition to the above mentioned demands, our requirements were as follows: (1) for multi-compounds membranes, different phases types have been observed (Dietrich et al., 2001; Korlach et al., 1999). Depending on the lipid part, different segregation behaviour in these phases could be expected. In consequence, this part of the molecule had to be interchangeable. (2) The lipid double fatty chain had to remain physiological, therefore, no fluorophore were expected in this hydrophobic part. (3) Distance and orientation (both between biotin and fluorophore or between biotin or fluorophore and the double lipid part, i.e. the GUV bilayer) could be changed in order to study the consequences of geometrical constraints for binding motor to the membrane, in a word, structure/properties relationships.

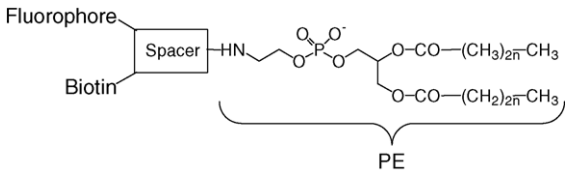

Fig. 1. Structure of fluorophoryl-biotinyl-PE.

We therefore decided to design our own phospholipids so that the fluorophoryl-biotinyl-PEs presented in Fig. 1 meet the above-mentioned requirements.

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

Fmoc-Gly-*O*-Wang resin **1**, Fmoc-L-Lys(Biotin)–OH and PyBOP were purchased from Novabiochem (France Biochem, Meudon, France). HBTU was from Senn Chemicals (Dielsdorf, Switzerland) and HOBt from Acros Organics (Geel, Belgium). Piperidine, diglycolic anhydride, DIEA, diaminopropane, ninhydrin, TMCR, TNBS, TFA, anisole and DSPE were purchased from Sigma–Aldrich (St-Quentin Fallavier, France) and used without further purification. All solvents used were of analytical grade and purchased from Sigma–Aldrich.

TNBS and ninhydrin colorimetric tests for amine detection were conducted using the methodology described in the 2002–2003 Novabiochem catalog (respectively Hancock and Battersby, 1976; Sarin et al., 1970).

RP-HPLC analyses were performed using Shimadzu apparatus with an analytical C-18 column (4 mm × 250 mm) and a 0.05% TFA water–acetonitrile linear gradient at 1 mL min^{-1}, $\lambda = 215$ nm.

The formation and purity of compound **7** was assessed by TLC analyses using silica gel 60 plates with $CHCl_3/H_2O/NH_4OH$ 37% in water: 70/30/4 (v/v/v)